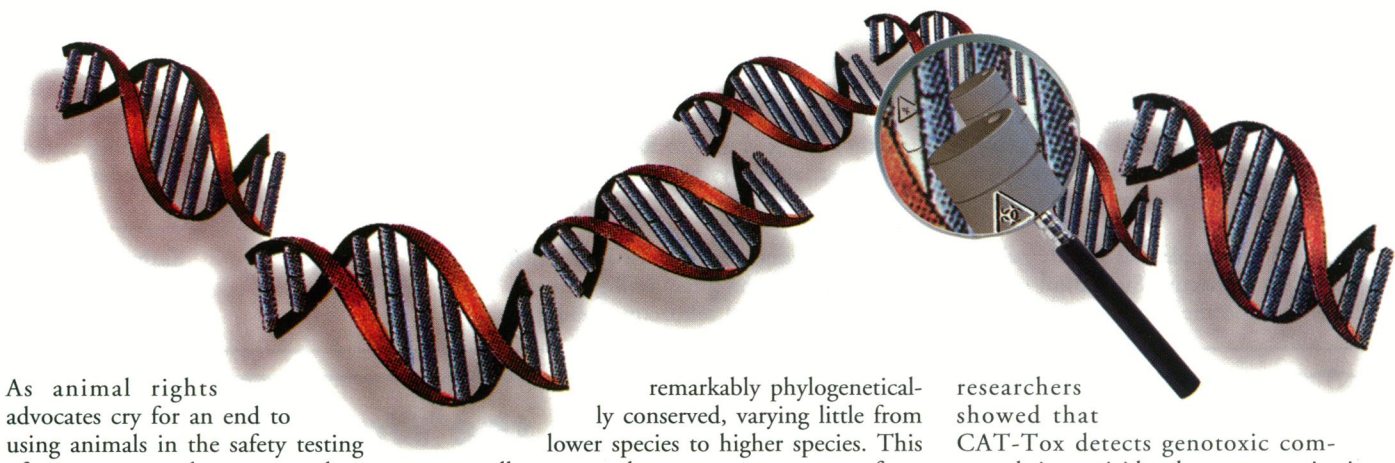
TRACKING TOXICANTS



As animal rights advocates cry for an end to using animals in the safety testing of consumer products, researchers worry about insuring that products are toxicologically safe. The gap between the two continues to narrow as scientists develop alternatives to animal testing.

At the forefront of the movment to reduce and replace experimental animals are the Stress Gene Assays created at Xenometrix, Incorporated, a five-year-old biotechnology firm in Boulder, Colorado. The Stress Gene Assays wed molecular biology with toxicology to identify toxic compounds. The new assays, which are cheaper, less time-consuming, and potentially far more informative at the mechanistic level than animal testing, should please both camps.

The Stress Gene Assays rely on the principle that cells respond in various well-defined ways when exposed to toxic substances. To defend themselves efficiently, nature endowed cells with "stress genes" to counteract toxicants either by detoxifying them or by repairing cellular damage caused by them. Stress genes are triggered by lipid oxidation, DNA damage, osmotic imbalance, protein misfolding, membrane perturbations, metals, heat shock, and numerous other conditions.

Researchers worldwide have isolated many stress genes from both prokaryotic and mammalian cells in the past 15 years. The most fundamental stress genes are remarkably phylogenetically conserved, varying little from lower species to higher species. This allows researchers to use stress genes from bacteria in assays aimed at predicting cellular damage to mammalian cells.

At Xenometrix, scientists place regulatory sequences of stress genes, called promoters, in front of a reporter gene that churns out an easily-measured color product. The lacZ region of the ß-galactosidase gene makes an ideal reporter because it degrades ortho-nitrophenyl-ß-D-galactopyranoside into the intensely yellow-colored product, ortho-nitrophenol, which can be measured optically with standard laboratory equipment at 420 nm. The activity of the stress gene promoter attached to lacZ is directly proportional to the concentration of the color product formed.

Xenometrix offers a line of Stress Gene Assays that target the needs of specific users. All the assays contain specific stress genes incorporated into a number of cell types, including bacteria and human liver, skin, and colon cell lines. For instance, the company's assay, sold by the trade name CAT-Tox(DNA), provides stress genes that respond to DNA damage in a human colon cell line. "The colon is a principle source of genotoxicity, because we ingest chemicals that cause DNA damage," says geneticist Mark Benjamin, director of scientific affairs at Xenometrix. At CEREGEN Environmental Health Laboratory (a division of Monsanto) in St. Louis, Missouri, researchers showed that CAT-Tox detects genotoxic compounds in pesticides that test negative in the Ames *Salmonella* assay, including camptothecin, 6-thioguanine, safrone, and urethane.

In the Xenometrix assay, CAT-Tox(Skin), stress genes in keratinocytes can be used by both cosmetic companies and pharmaceutical firms in designing dermally-delivered drugs. Skin patch drug-delivery systems, which are becoming more popular, carry the risk of causing irritation and inflammation. Pharmaceutical companies must find compounds that deliver drugs without producing local side effects.

Mary Kay Cosmetics is evaluating CAT-Tox(Skin) to eliminate cosmetic ingredients that irritate the skin. "Consumers want skin products that never sting or itch, not even on dry, winter skin," says biochemist Myra Barker, chief scientific officer at the Dallas, Texas-based company. Finding such "small perturbations in skin equilibrium requires really sensitive assays," says Barker. Screening ingredients with *in vitro* assays may identify and eliminate irritants prior to expensive human trials.

Mary Kay Cosmetics, which has not conducted animal testing on its finished products since 1989, is "way beyond the issue of replacing animals. We want *in vitro* tests that give us answers that we

never had the tools to obtain before," says Barker. In addition, consumers could benefit, as the current high cost of human testing restricts the flow of new cosmetic ingredients into the marketplace.

The Stress Gene Assays come as standard 96-well microtiter plates, requiring only microgram quantities of a test compound. To test one compound at seven different concentrations in triplicate takes about six hours in some assays, whereas others need overnight exposure to toxicants. Xenometrix also provides computer software that converts optical density values directly into gene activity. The results are conveniently represented as a "fingerprint," or histogram, showing the pattern of stress gene activation relative to toxicant dose. The assays, which range in price from $1,000 to $3,000, contain 8–16 stress genes.

Toxicology Improved

Traditionally, toxicology studies assume that laboratory animals predict human risk unless there is information to demonstrate species differences. Such extrapolation is sometimes inaccurate, because species may differ in responses to toxicants. In addition, animal toxicology often uses histopathology as an endpoint, which is labor-intensive, and may fail to detect some early biological changes, such as mutations or oxidative damage. Stress Gene Assays, however, can reveal the toxic potential of chemical compounds and pinpoint specific processes involved. And stress genes respond at concentrations of chemicals below those needed to cause histopathological damage. There are limitations to the assays using stress genes, however, particularly that they do not provide information on absorption, distribution, metabolism or excretion of chemicals in an intact whole animal.

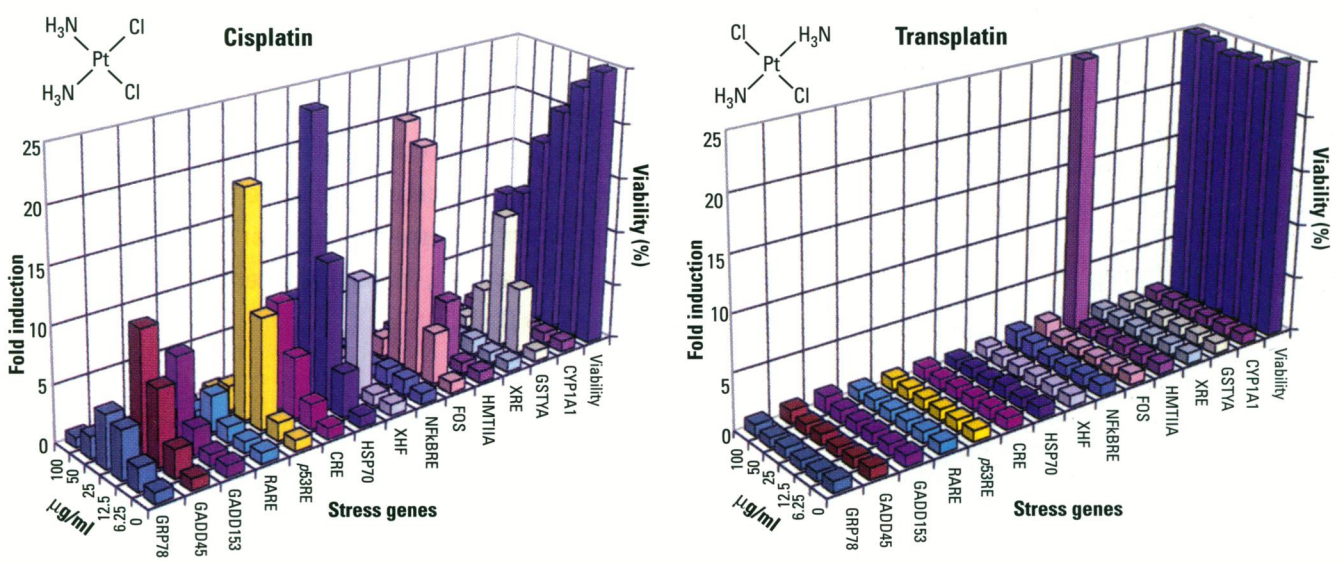
Xenometrix scientists have verified the specificity of their assays by testing them with well-known toxicants in the laboratory. They have found that toxic compounds, such as paraquat, methylmercury, and mitomycin C, trigger expected stress genes. For example, paraquat, a powerful generator of superoxide radicals, induces stress genes sensitive to redox changes and DNA damage. Mercuric chloride specifically induces the stress gene that codes for mercury reductase. And mitomycin C, known to be severely toxic, induces several kinds of stress genes, suggesting that it affects multiple metabolic pathways and cellular functions.

Burlington Research Incorporated, an environmental research laboratory based in Burlington, North Carolina, determines toxicants in industrial wastewater from textile mills, metal industries, rubber manufacturing, and poultry plants with Stress Gene Assays. "We're compiling stress gene responses to 25 known persistent aquatic toxicants that escape conventional biological treatment of wastewater," says Samuel Moore, president and CEO at Burlington Research. The unique stress gene pattern generated for each substance is recorded in a database that can be read by pattern recognition software developed at Burlington Research. Eventually, a toxicant's fingerprint pattern will make it possible to trace pollutants "way back up the sewer pipe to the source, just like a detective following a trail," says Moore.

Moreover, Stress Gene Assays cut the time and cost of testing water samples. Standard biological procedures require monitoring the reproduction or growth rate of crustaceans or minnows for at least 7 days. And even if the creatures fail to reproduce or thrive (indicators of chemical exposure), the specific pollutant is not always identified. Narrowing down the culprit toxicant may require further labor-intensive extraction or separation procedures, followed by another 7-day biological test. Burlington Research reduced its costs from $5,000 (and 40 hours of labor) to $2,000 (and 16 hours labor) per sample with the Xenometrix system. "The traditional technology is too slow, too expensive, and too ambiguous," says Moore.

Addressing the Future

So far, no company has abandoned animal testing and replaced it with Stress Gene Assays. "Our technology is still new, and has not become mainstream yet," says Benjamin. The assays,which are just two years old and some of which are still being fine-tuned, take time to learn and interpret. Unlike standard laboratory assays that give black-or-white answers, such as



Toxic fingerprints. Xenometrix histograms show induction of stress genes in liver cells treated with the toxic agents cisplatin and transplatin. The cisplatin graph shows DNA-damage responses (GADD45, GADD153, and p53RE), responses to protein perturbation (HSP70) and oxidative stresses (NFkBRE and FOS), and significant cytotoxicity (Viability). The transplatin graph shows no cytotoxicity across this concentration range, but induces high levels of metallothionein (HMTIIA).

positive or negative results, the Stress Gene Assays give "many thousands of shades of gray," says Benjamin, and the "data are not always easy to interpret." To address this issue, Xenometrix is developing a database of fingerprints of known toxic compounds that customers can turn to for reference.

Some potential users may find the assays' $1,000–3,000 price tags too costly. But, Benjamin explains, because an assay provides 16 endpoints (stress genes) for one or two toxicants in triplicate and ready for statistical analysis, he believes the amount of data generated justifies the expense. Also, unlike the Stress Gene Assays, "many animal studies are not acute," says Benjamin. For example, rats, which cost $20–$200 each, must be housed and fed for 90 days to 2 years, depending on the goal of a toxicology study.

As researchers at biotechnology, pharmaceutical, cosmetic, chemical, and environmental companies gain more confidence in the abilities of Stress Gene Assays to generate useful data, "they will replace animals," predicts Benjamin. Eventually, a stress gene pattern will fingerprint a chemical's toxic potential, much as nuclear magnetic resonance imaging or gas chromatography identifies its structural groups. Based on stress gene fingerprints, toxicologists may be able to eliminate compounds from further costly development or design new compounds with acceptable toxicity. An early stress gene advocate, Moore says, "Stress gene technology will be the toxicity characterization method of the future."

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Suggested Reading

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